DROUGHT STRESS

Evaluation of Common Bean for Drought Tolerance in Juana Diaz, Puerto Rico

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Abstract

Drought tolerance is an increasingly important trait in common bean (*Phaseolus vulgaris* L.) due to the reduction in water resources, a shift in production areas and increasing input costs. The objective of this study was to evaluate 29 genotypes for drought tolerance under drought stress (DS) and reduced stress treatments in Juana Diaz, Puerto Rico. The use of DS and reduced stress treatments facilitated the identification of drought tolerant germplasm that also had good yield potential under more optimal conditions. Based on the results of seed yield under DS and reduced stress conditions, and DS indices, including the geometric mean (GM), stress tolerance index (STI) and percent yield reduction (YR), genotypes were identified with greater yield potential under the tested environment. Based on average GM over the 2 years, the superior common bean genotypes identified were SEA 5, G 21212, A 686, SEN 21 and SER 21. These genotypes performed well in both years and under both treatment conditions and thus may serve as parents for DS improvement and genetic analysis.

Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important food legume (Broughton et al. 2003); however, drought stress (DS) results in significant seed yield reductions in 60 % of global bean production areas (White and Singh 1991). Increasing competition for production area has resulted in a move of bean acreage to more marginal zones often associated with increased abiotic stress (Porch et al. 2008). The majority of common beans are produced in the Midwestern and Western areas of the US, while DS impacts over 50 % of the cropped area in the Western US (Cook et al. 2004). Considering mounting pressure on limited water resources, and additional factors such as climate change and increasing household water use, water threatens to become a progressively scarce resource. Given the high consumption rates of water by agriculture, constraints on water resources can be mitigated through the genetic improvement of DS tolerance in crop species. Genetic improvement, based on using existing genetic variability in the species, is founded on the evaluation and selection of drought tolerant germplasm and on the understanding of the physiological and genetic responses to abiotic stress.

The duration and severity of DS in common bean determines the level of seed yield reduction (Singh 1995). A number of indirect techniques have been used for the evaluation of drought tolerance; however, seed yield is the most reliable indicator because it directly represents the harvestable product (White et al. 1994a,b, Ramirez-Vallejo and Kelly 1998). Seed yield reductions due to drought are further increased as a result of interactions with other sources of stress, such as high temperature, disease and soil conditions (Ramirez-Vallejo and Kelly 1998). The effects of diseases are important under DS, such as root rot caused by *Fusarium solani*, *Pythium ultimum* and *Rhizoctonia solani*, as well as ashy stem blight, caused by *Macrophomina phaseolina*. Other abiotic stresses, such as high temperature stress, can also interact and compound the effects of DS, but can be evaluated using the same stress indices (Porch 2006). Thus, multiple stress tolerance is an important consideration for DS breeding (Beebe et al. 2008). Reproductive development is particularly sensitive to drought, resulting in increased abortion and abscission of buds, flowers and pods, and a reduction in seed yield (Calvache et al. 1997, Muñoz-Perea et al. 2006). Due to the importance of reproductive development in the DS response, higher water requirements during reproductive development, and the occurrence of terminal DS in production areas worldwide, germplasm evaluation in common bean is commonly conducted through the application of DS between pre-flowering and physiological maturity. This DS is applied intermittently (Muñoz-Perea et al. 2006) or as a terminal treatment with no water applied during this period (Beebe et al. 2008).

Evaluations of germplasm for drought tolerance have identified key sources of tolerance. High levels of drought tolerance have been found among the Mesoamerican gene pool in races Durango (Singh et al. 1991, Acosta-Gallegos et al. 1999, Terán and Singh 2002, Muñoz-Perea et al. 2006), Mesoamerica (Rao 2001, Terán and Singh 2002, Beebe et al. 2008), and Jalisco (Terán and Singh 2002). The Durango race, from the dry Mexican highlands, has been widely used for breeding for drought tolerance, contributing both higher seed yield and harvest index (Singh et al. 1991), while Mesoamerica may contribute improved seed filling traits (Rao 2001), in addition to seed yield under DS (Beebe et al. 2008). The intercrossing of these two races has resulted in improvements in stress tolerance (Terán and Singh 2002, Frahm et al. 2004, Beebe et al. 2008). Thus, the combination of traits associated with drought tolerance from both gene pools can result in significant gains in seed yield in drought-prone environments (Beebe et al. 2008). Germplasm development has resulted in the release of a number of lines tolerant to DS and has led to a better understanding of the genetics of this trait. Drought tolerance, measured as seed yield, is an additive and quantitative trait with significant interaction with the environment (White et al. 1994a, b). A range of heritabilities, 0.09–0.80, were found for DS depending on environmental and genetic factors (White et al. 1994a, b, Singh 1995, Schneider et al. 1997). Thus, due to high genotype by environment interaction, the evaluation of germplasm for drought tolerance in the target environment is important for the selection of genotypes for incorporation as parents in breeding programs.

The objectives of this study were to determine the seed yield response to DS in germplasm derived primarily from the Mesoamerica race of common bean or derived from the hybridization of the Mesoamerica and Durango races. Most of the genotypes tested have shown some level of drought tolerance in previous studies. The trials were carried out using two levels of water stress, DS and reduced stress treatments.

Materials and Methods

Twenty-nine genotypes were selected for this study. The genotypes A 686, A 774, BAT 477, RAB 655, SEA 5, SEA 15, SEC 5, SEN 3, SEN 10, SEN 20, SEN 21, SEN 22, SER 10, SER 16, SER 21, SER 22, SER 26, VAX 1, VAX 2, VAX 3, VAX 4 and VAX 6 were released as germplasm from CIAT (International Center for Tropical Agriculture) for tolerance to abiotic or biotic stress. The SEC, SEN and SER lines are recent releases for drought tolerance in the cream, black and small red market classes, respectively, while the SEA lines were previously released for drought tolerance. Many of these lines are derived from crosses between the Durango and Mesoamerica races (S. Beebe, personal communication). The VAX lines are resistant to common bacterial blight (Singh and Munoz 1999) and possess additional stress tolerance. A 774, BAT 477, G 21212 (a landrace) and RAB 655 have multiple stress tolerance, including tolerance to drought and low soil phosphorus (CIAT 2004), while 'ICA Pijao' is a Colombian cultivar used as a drought susceptible check. 'Kodiak' (Kelly et al. 1999) and 'Maverick' (Grafton et al. 1997) are pinto cultivars from the Durango race, while 'Morales' is a small white bean cultivar (Beaver and Miklas 1999) that served as the local check. 'Tio Canela' is a small red bean cultivar (Rosas et al. 1997) with heat tolerance and TB1 is a cultivated tepary bean (*P. acutifolius* L.) that was used as a drought tolerant check.

The field experiments were conducted at the Experimental Station of the University of Puerto Rico in Juana Diaz, PR. The station is located at 18°01′N latitude and 66°22′W longitude, at an elevation of 21 m above sea level, and in a semi-arid climatic zone (Goyal and Gonzalez 1989). The average monthly rainfall during January, February and March are 19.8, 18.3 and 21.8 mm, respectively. The mollisol soil is classified as a San Anton Clay Loam, with a 0.38 $cm^3\ cm^{-3}$ field capacity (FC) and a 0.18–0.20 $cm^3\ cm^{-3}$ wilting point.

The field experiments were planted on 17 January 2007 and on 25 January 2008. Climatic data were recorded during crop development (Table 1). A split-plot design was used with water level serving as the whole-plot treatment factor and genotype as the split-plot treatment factor. The whole-plot was divided into two treatments, DS and reduced-stress (RS), where the RS treatment, as compared to the commonly used term, non-stress treatment, indicates that other stressors, including drought, were present in the treatment. Five replications of each

Table 1 Climatic conditions during drought stress and reduced stress experiments in Juana Diaz, Puerto Rico in 2007 and 2008

Year	Treatment	Irrigation (mm)	Rainfall (mm)	Number of days $ET_o > 5$ (mm $days^{-1}$)	Mean max. temp. (°C)	Mean min. temp. (°C)	Number of days max. temp. >32 °C	Number of Days min temp. >22 °C	Mean relative humidity (%)
2007	Drought stress	248.3	53.7	15	30.1	20.6	2	13	64.4
2007	Reduced stress	379.7							
2008	Drought stress	195.0	29.2	7	31.1	18.1	11	0	57.4
2008	Reduced Stress	430.0							

genotype were planted in a randomized complete block design (RCBD) within each whole-plot treatment. Single, 4 m length plots were planted with 1 m spacing between rows, with a target plant density of 130 000 plants per hectare. Drought stress was applied intermittently in 2007 and 2008 starting at the beginning of reproductive development through harvest. Irrigation was applied in the DS treatment when the soil moisture content was reduced by 75 % of the FC. The plants showed mid-day wilting symptoms between irrigations during the complete period of reproductive development. Irrigation was measured using a cumulative electronic digital flow meter (GPI Inc., Conyers, GA, USA), and was recorded at the beginning and end of each irrigation event and was generally applied twice per week using a drip irrigation system. Water application was monitored using access tubes installed at 20 and 40 cm depths and the volumetric moisture content was measured with a PR2 sensor (Delta-T Devices Ltd, Cambridge, UK) profile probe. In 2007, the DS treatment received 302 mm total water and the RS treatment received 433 mm total water (Table 1). In 2008, the DS treatment received 224 mm total water and the RS treatment received 459 mm total water. Other agronomic practices were consistent throughout the experiments.

Climatic data were collected using two weather stations positioned within the field experiments. Actual evapotranspiration (ET) was derived from estimates of canopy and aerodynamic resistance (Ramírez-Builes 2007) during the whole growing season using the generalized Penman-Monteith model (Monteith and Unsworth 1990). Reference ET was derived using the United Nations Food and Agriculture Organization method (Allen et al. 1998). Seed yield reduction under stress (YR, $1 - Y_s/Y_p$; Fischer and Maurer 1978), geometric mean (GM, $(Y_s \times Y_p)^{1/2}$; Fernandez 1993), stress tolerance index (STI, $(Y_p \times Y_s)/X_p^2$; Fernandez 1993), and the drought intensity index (DII, $1 - (X_s/X_p)$; Fischer and Maurer 1978) were determined. The DS and reduced stress seed yield data (kg ha^{-1}) from the trials in this study were used for yield under stress (Y_s) and potential yield under non-stress (Y_p) variables, respectively. X_s and X_p were the mean yield of all genotypes per trial under stress and non-stress conditions, respectively.

The statistical analyses were conducted using MINITAB (State College, PA, USA) and SAS (SAS Institute, Cary, NC, USA). A mixed model was used for data analysis with year and replication considered as random variables, while treatment and genotype were considered fixed effects. The homogeneity of error variances for both years was tested (Bartlett) after data analysis was completed for each year separately, followed by a combined data analysis. Least significant difference (LSD, $\alpha = 0.05$) was used to compare the mean of the genotype seed yield data by year and by treatment. To analyse the results in graphical form, a plot of yield under stress and reduced stress conditions was completed. Vertical and horizontal lines in the plot were placed to represent trial mean yield under DS (x-axis) or reduced stress (y-axis) conditions and thus to divide up the germplasm into response categories.

Results

After testing for homogeneity of error variance, a combined data analysis for 2007 and 2008 was completed (Table 2). The statistical analysis showed that genotype, treatment * genotype, and replication effects were significant, while the treatment ($P = 0.078$) and year ($P = 0.098$) effects were not significant, which was possibly due to the low number of degrees of freedom not providing the adequate levels of precision to detect a significant difference. The other effects not mentioned were not significant. The overall response to drought was as expected in the control genotypes. The drought tolerant check, TB1 (tepary bean), had the highest seed yield under DS in both years, while the drought susceptible check, ICA Pijao, showed lower yield potential under DS, but also under the reduced stress treatment (Table 3).

The seed yield ranged from 77 to 494 kg ha^{-1} under DS and from 673 to 1051 kg ha^{-1} under RS in 2007, and from 578 to 817 kg ha^{-1} under DS and from 942 to 1693 kg ha^{-1} under RS in 2008 (data not shown). However, average seed yields were higher in 2008 than in 2007, specifically 57 % higher under DS and 40 % higher under RS in 2008. Higher levels of daily ET were identified during 2007, 15 days of $ET_o > 5$ mm day^{-1}, while there were only 7 days of $ET_o > 5$ mm day^{-1} in 2008. In

Table 2 Combined analysis of variance for yield (kg ha^{-1}) for field trials in 2007 and 2008 in Juana Diaz, Puerto Rico

Source	DF	MS adjusted	F	P
Year	1	34 090 248	37.05	0.098
Treatment	1	59 690 096	65.98	0.078
Year * Treatment	1	904 706	1.89	0.188
Replication (Year * Treatment)	16	472 952	15.96	0
Genotype	28	158 581	3.06	0.002
Year * Genotype	28	51 873	1.42	0.178
Treatment * Genotype	28	68 689	1.88	0.050
Year * Treatment * Genotype	28	36 450	1.23	0.197
Error	448	29 643		
Total	579			
CV[1]	20.7			

[1]Coefficient of variation for the combined data analysis.

2007, the seasonal reference ET was 263 mm, while the ET was 145 mm under stress and 178 mm in the reduced stress treatment; in 2008, the reference ET was 299 mm, while the ET was 121 mm under DS and 198 mm in the reduced stress treatment (data not shown). Under the DS treatment in both years, the soil water content was maintained close to the wilting point. The DII, based on the difference between the stress and reduced stress treatments within each year, was similar between the 2 years, 0.64 in 2007 and 0.50 in 2008.

The common bean genotypes varied significantly in seed yield response, while the tepary bean (TB1) was clearly more tolerant to drought than the common bean lines with at least 30 % higher yield under DS (Table 3). Among the common bean genotypes in the combined analysis, SER 21 showed the highest seed yields under DS, followed by SER 16, SEA 5, SEA 15 and G21212. The five superior common bean genotypes for each yield index were also identified. SEA 5, G 21212, SER 21, SEN 21 and A 686 showed the highest STI in the combined analysis, while SER 22, SER 21, SER 16, Maverick and SER 26 had the lowest average seed yield reduction (YR). SEA 5, G 21212, A 686, SEN 21 and SER 21 had the highest GM in the combined analysis.

A plot of seed yield (kg ha^{-1}) under stress and non-stress conditions for each genotype (Fig. 1), organized into quadrants, was completed for the combined 2007 and 2008 yield data. Ten genotypes, A 686, G 21212, Kodiak, Morales, SEA 5, SEA 15, SEC 5, SEN 10, SEN 21 and TB1, showed higher seed yield (upper right quadrant) under both treatments. An additional six genotypes, Maverick, SER 10, SER 16, SER 21, SER 22 and SER 26, showed higher yield under DS (lower right quadrant). However, SER 22 showed low seed yield potential under reduced stress conditions. ICA Pijao, a drought susceptible check, had low to average yields in both treatments (lower left quadrant).

Table 3 Mean yield (kg ha^{-1}) under reduced stress (RS) and drought stress (DS), and calculated drought stress indices[1] including, geometric mean (GM), stress tolerance index (STI), and percent yield reduction (YR) for 29 genotypes derived from separate and combined analysis of data from field trials in 2007 and 2008 in Juana Diaz, Puerto Rico

	Combined analysis				
Genotype	RS	DS	GM	STI	YR
A 686	1320	549	845	0.51	0.61
A 774	1058	490	710	0.36	0.57
BAT 477	1259	474	747	0.40	0.66
G 21212	1300	563	854	0.53	0.58
ICA Pijao	1126	389	656	0.31	0.68
Kodiak	1236	562	833	0.51	0.56
Maverick	1079	510	742	0.42	0.52
Morales	1232	525	791	0.46	0.60
RAB 655	1064	332	567	0.23	0.73
SEA 5	1318	567	864	0.54	0.58
SEA 15	1215	567	830	0.51	0.54
SEC 5	1235	519	799	0.48	0.59
SEN 3	1058	471	706	0.37	0.55
SEN 10	1167	545	796	0.47	0.55
SEN 20	1136	413	681	0.33	0.66
SEN 21	1270	555	839	0.51	0.57
SEN 22	1070	469	708	0.36	0.57
SER 10	1145	557	793	0.46	0.54
SER 16	1105	586	802	0.49	0.48
SER 21	1131	620	837	0.52	0.46
SER 22	859	536	679	0.39	0.37
SER 26	1123	552	784	0.46	0.52
TB1	1312	873	1067	0.87	0.30
Tio Canela	1157	444	711	0.36	0.64
VAX 1	1211	348	618	0.27	0.74
VAX 2	1164	419	681	0.33	0.69
VAX 3	1053	440	672	0.33	0.61
VAX 4	1009	490	700	0.35	0.54
VAX 6	964	408	622	0.28	0.61
Mean	1151	509	756	0.43	0.57
LSD (0.05)[2]	214	214			

[1]$GM = (Y_s \times Y_p)^{1/2}$; $STI = (Y_p \times Y_s)/X_p^2$; $YR = 1 - Y_s/Y_p$. The genotype specific variables include yield under stress (Y_s) and potential yield under non-stress (Y_p). X_s and X_p are the mean yield of all genotypes per trial under stress and non-stress conditions, respectively.
[2]LSD at P = 0.05 for comparison of means between genotypes.

Discussion

The intermittent DS applied during reproductive development in this study closely mirrors the DS encountered in many agricultural production zones, resulting from reduced irrigation and/or limited rainfall in rainfed regions.

In this study, significant genotype and replication effects were found, as well as a significant genotype by treatment interaction, which is likely due to the differential response of the genotypes to variable levels of DS. The use of DS and RS treatments allowed for the

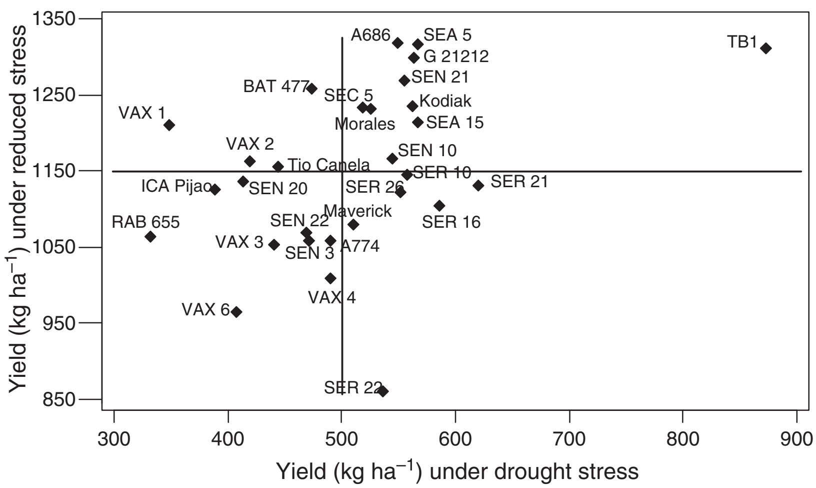

Fig. 1 Comparison of 29 common bean genotypes for combined 2007 and 2008 mean yield (kg ha^{-1}) under intermittent drought stress and reduced stress treatments in a split-plot experiment from field trials conducted in Juana Diaz, Puerto Rico. Vertical and horizontal lines represent trial mean yield under drought stress (x-axis) or reduced stress (y-axis) conditions. Resulting quadrants are an indication of yield response under drought and reduced stress conditions.

comparison of seed yield response under different levels of DS. The DII indicated that there were similar reductions in yield due to the stress treatment in both years. Because the treatments were planted side by side in a split-plot design, the biotic and abiotic conditions were similar within each year, such as high temperatures and the incidence of pests and diseases, but varied across years. However, in this study, the general genotypic response to the treatments was relatively consistent year to year, resulting in relatively consistent rankings of genotypes. Because of frequent variability in climatic conditions year to year and the quantitative nature of the drought response, it is important to test for drought response over multiple years and under both stress and reduced stress treatments. In addition, significant genotype by environment interaction often makes extrapolation of genotypic results from one location to another less effective.

The seed yields under stress in this study are comparable to other drought experiments conducted in tropical and sub-tropical environments (e.g. Frahm et al. 2004, Beebe et al. 2008), while the yields under non-stress or reduced stress conditions were lower in this study. Seed yields in 2007 were substantially lower than in 2008. Low seed yields in both trials in 2007 were likely due to inadvertent DS applied to both the DS and RS treatments at the beginning of flowering as a result of delayed watering, as well as high daily ET, and higher night time temperatures. Root rot, stem rots and other diseases were not significant. Although there were a greater number of days with higher day time temperatures in 2008, the number of days with high night time temperatures was greater in 2007. Average night time temperature was 2.5 °C higher in 2007 vs. 1.1 °C higher for daytime temperatures in 2008. Common bean is especially sensitive to high night time temperatures during reproductive development (Ofir et al. 1993) resulting in seed yield reduction. Higher daily ET (>5 mm day^{-1}) was found in 2007; however, based on seasonal ETs, high levels of stress were present in both years and resulted in incipient plant wilting in the early afternoon.

The majority of the genotypes chosen for this study were developed for tolerance to drought. Thus, relatively high levels of DS tolerance were expected among the genotypes tested. Considering the similarity in the results between GM and STI in this study and the tested efficacy of GM for the evaluation of drought tolerance (Schneider et al. 1997), GM was considered the superior index for evaluation of germplasm under DS and RS conditions. Based on field performance during two years in Puerto Rico, several genotypes may serve as good sources of drought tolerance for genetic improvement. Specifically, SEA 5, G 21212, A 686, SEN 21 and SER 21 were selected based on GM in the combined analysis. Several of these genotypes resulted from inter-racial crosses of Durango and Mesoamerica types, including SEA 5, SEN 21 and SER 21. In addition, SEA 5 and G 21212 were previously found to possess drought tolerance and deep tap roots (CIAT 2004) which may have played a role in their performance in these trials. Morales, the local check, showed good performance under both treatments as well as the highest seed yield potential in 2007. ICA Pijao, the susceptible check, had low yields under DS and average yields under reduced stress treatments. Kodiak consistently yielded well under both treatments, however, Kodiak was previously classified as drought susceptible (Singh 2007) showing relatively low seed yields under both non-stress and drought-stress treatments in that study. Kodiak may be well-adapted to Puerto Rico given that it underwent two seasons of selection in Isabela, Puerto Rico (Kelly et al. 1999).

In conclusion, given the difficulty in applying similar levels of abiotic stress in consecutive years and in controlling other sources of stress, the use of multiple levels of stress treatment within each year in multi-year trials is useful for the identification of drought tolerant germplasm. Genotypes were identified that provide consistent yield under both stress and reduced stress conditions and that can serve as important sources of drought tolerance for breeding, and genetic analysis. Due to variability in the levels of stress and the interaction with other confounding stressors in drought prone environments, multiple stress tolerance is critical in germplasm and cultivar development for drought tolerance.

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